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(21) International Application Number: PCT/SE93/00502 (22) International Filing Date: 7 June 1993 (07.06.93) (30) Priority data: 243070 8 June 1992 (08.06.92) NZ (71) Applicant (for all designated States except US): KABI PHARMACIA AB [SE/SE]; S-751 82 Uppsala (SE). (72) Inventors; and (75) Inventors/Applicants (for US only) : GLUCKMAN, Peter, David [NZ/NZ]; 78 Lucerne Road, Remuera, Auckland 5 (NZ). MELLOR, David, James [NZ/NZ]; 159 Moonshine Valley Road, Aokautere RD1, Palmerston North (NZ).		(74) Agents: TANNERFELDT, Agneta et al.; Kabi Pharmacia AB, S-112 87 Stockholm (SE). (81) Designated States: AU, CA, FI, JP, NO, NZ, RU, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: USE OF GROWTH FACTOR IGF-I AND/OR IGF-II (57) Abstract <p>The present invention relates to the use of IGF-I and/or IGF-II or effective analogues thereof for the manufacture of a medicament adopted for oral or any gastrointestinal route of administration for prevention or treatment of pancreatic disorders and insufficiency. It also relates to composition comprising exogenous human or animal IGF-I and/or IGF-II or effective analogs thereof comprising foodstuff for oral administration, preferably in admixture with artificial or natural milk or colostrum.</p>		

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USE OF GROWTH FACTOR IGF-I AND/OR IGF-II

The present invention relates to the use of IGF-I and/or IGF-II or effective analogues thereof for the manufacture of a medicament adopted for oral or any gastrointestinal route of administration for prevention or treatment of pancreatic disorders and insufficiency. It also relates to composition comprising exogenous human or animal IGF-I and/ or IGF-II or effective analogues thereof comprising foodstuff for oral administration, preferably in admixture with artificial or natural milk or colostrum. The invention may be applied both in man and in animals.

INTRODUCTION AND PRIOR ART

Insulin-like growth factor¹ (IGF-I) and insulin-like growth factor 2 (IGF-II) are peptides present in plasma and other body fluids. They show 64 % homology of their primary sequences and comprise 70 and 67 amino acids respectively, including 3 disulphide bonds. They can stimulate growth of a wide range of cell types. IGF-I but not IGF-II mediates the effects of growth hormone on skeletal growth. Both IGF-I and IGF-II have been purified from human plasma and their complete amino acid sequences are known. Sequences with extensive homologies to human IGF-I and IGF-II are present in IGF-I and IGF-II purified from plasma of other species. IGF-I and IGF-II have both systemic and local effects and appear mostly associated with different specific binding proteins, six of which are sequenced and are termed IGFBP1, IGFBP2, IGFBP3, IGFBP4, IGFBP5 and IGFBP6. These appear to modulate the biological functions and availability of IGF-I and IGF-II in both a positive and negative manner. Analogues with changed affinities for the binding proteins have been produced and changes of biological activities related to sequence variation have been found. Both IGF-I and IGF-II appear to act mainly by interactions with the IGF-type 1 receptor exposed on

the outer surface of plasma membranes in many different cell types - however relative specificity of action may be found because of the influence of binding proteins. Further IGF-II may have distinct actions as it alone binds to a distinct and unrelated type 2 receptor also found on cell membranes. Further binding of both IGF-I and IGF-II to insulin receptors also seems to be of importance.

Because of the scarcity of purified plasma IGF-I and IGF-II there was a great necessity to develop methodology for the commercial scale production of IGF-I and IGF-II. Nowadays such large scale production can readily be achieved by using recombinant DNA techniques. As a result of studies with preparations of recombinant DNA derived IGF-I it has been demonstrated that it promotes skeletal growth and skeletal muscle protein synthesis. Moreover IGF-I is also effective for the treatment or prevention of catabolic states (Swedish patent application SE 9002731-9). IGF-II has also been shown to antagonise some metabolic actions of IGF-I (Koea et al Endocrinology (1992). 130, 2423-2425).

Ballard et al , WO 91/12018 have disclosed the therapeutic use of IGF-I for gastrointestinal disease or the treatment of the shortened gut after surgery. Ballard provides no evidence of activity or effects on pancreatic size or growth following oral administration.

It has previously been demonstrated that both type 1 and type 2 IGF receptors are present in the gastrointestinal tract and that oral IGF-I and IGF-II affect jejunal enzymes following repeated administration in older suckling rats, but no effect on intestinal growth was observed. (Young et al. Digestion 46 , 1990, Suppl. 2, 240-252). It has also been reported that systemically (i.e. subcutaneous or intramuscular or intravenous) administered IGF-I can increase gastrointestinal weight, but pancreatic growth was not assessed.

Heinze-Erian et al, Endocrinology, Vol 129, No 4, 1769, 1991 reports that there is an essential role for both IGF receptors in the regulation of cell mitogenesis and growth.

Rivard N et al, Regul-Pept 1991, Jun 11, Vol 34(1), 13-23 reports that intravenous injected IGF-I may have an effect on pancreatic and duodenal cell content of somatostatin and Grey V et al, Mol-Cell-Endocrinol, 1991, Mar, Vol 75 (3), 221-7 suggest that IGF-II/M6P receptor levels are altered in the intestinal epithelium following gut resection.

Earlier studies have shown that there are IGF as well as insulin receptors on the pancreas cells, but as there are IGF receptors on all cell types, this does not in any way suggest an action or a potential specificity of action.

There was thus not a priori reason to believe that IGF-I or IGF-II might be efficacious in the growth of the pancreas.

No effect of IGF-I or II on exocrine pancreatic function has been previously suggested and no studies of the effects of oral administration of the IGF's on pancreas have been previously suggested.

There is a need for a medicament adopted for oral or any gastrointestinal route of administration for prevention or treatment of pancreatic disorders and which also can be used for promoting pancreatic growth which is abnormal in the growth retarded animal.

It has now surprisingly been found that IGF-I and/or IGF-II or effective analogues thereof can be used for the manufacture of such a medicament.

THE INVENTION

The invention relates to the use of IGF-I and/or IGF-II or effective analogues thereof for the manufacture of a medicament adopted for oral or any gastrointestinal route of administration for prevention or treatment of pancreatic disorders.

The medicament also promotes pancreatic growth and is useful in the treatment of pancreatic insufficiency such as in intrauterine growth retardation, post partial pancreatectomy, cystic fibrosis and following pancreatitis.

Human or animal IGF-I or IGF-II can be used and may be given singly or in combination with each other or with other growth factors such as epidermal growth factor (EGF) for enhancing or improving the desired effect(s) of IGF-I, IGF-II or its effective analogues.

The invention relates to a composition comprising exogenous human or animal IGF-I and/ or IGF-II or effective analogues thereof comprising foodstuff for oral administration, preferable artificial or natural milk or colostrum.

In animal either recombinant human IGF-I and/or IGF-II or IGF-I or IGF-II of other species (e.g. bovine, porcine) may be used either as an oral drench or as a supplement to artificial liquid or solid feeds.

Regarding the use for prevention or treatment of pancreatic disorders, the following uses are pointed out:

1) Promotion of pancreatic growth in man.

In diseases associated with inadequate exocrine pancreatic function leading to fat malabsorption e.g. cystic fibrosis or in infants following partial/complete pancreatectomy for islet cell hyperplasia. In growth retarded infants who have disproportionately reduced pancreatic size.

2) Treatment of exocrine pancreatic deficiency

In diseases such as cystic fibrosis, postpancreatectomy.

3) Promotion of pancreatic development in growth retarded newborn.

4) Treatment in animals

Promotion of growth of the pancreas to restore intestinal function of the growth retarded animals.

Preferably in man human IGF-I or IGF-II singly or in combination is used. The dose given could be 1 to 100 $\mu\text{g/kg}$ /body weight per day in man and 1-1000 $\mu\text{g/kg}$, per day in animals. The preferred route of administration is by mouth either in aqueous buffer or other pharmacological composition or added to artificial feed, artificial or natural milk. Alternatively it may be installed more distally in the gastrointestinal tract for example by nasogastric tube or by duodenal tube.

In animal either recombinant human IGF-I or IGF-II of other species (e.g. bovine, porcine) may be used either as an oral drench or as a supplement to artificial liquid or solid feeds.

The claimed composition can be used as a supplement to foodstuff.

The foodstuff is preferably milk for the prevention or treatment of pancreatic disorders and for the promotion of pancreatic growth. Such a composition will at the same time promote nutrition, growth and reduce the impact of gastrointestinal infection.

This is desirable in human infants with growth failure, prematurity or where there is difficulty in establishing oral feeding.

The use is particularly desirable in infant animals from large litters, on artificial feeds or where there is growth retardation present.

The invention also extends to a nutritionally acceptable composition for the supplementation of natural or artificial milk formula for human or animal use such that similar amounts of IGF-I and/or IGF-II or analogue are provided.

The peptide may be present in amounts sufficient to provide a dose rate of approximately 1 to 100 $\mu\text{g/kg}$ body weight per day, preferably 1-10 $\mu\text{g/kg}$ per day in man and 1-1000 $\mu\text{g/kg}$ per day in animals.

DETAILED DESCRIPTION OF THE INVENTION

The preferred form of the invention will now be described with reference to the following non-limiting example.

EXAMPLE 1

In a study 11 pigs not otherwise treated, pancreatic chemistry was ascertained after sacrifice immediately after birth.

6 of these were of normal size and 5 were intra uterine growth retarded (IUGR) at birth (Table 1). IUGR piglets were defined as those at least 2 standard deviations (SD) below the mean, whereas normal piglets had at birth weight within 1 SD of the mean.

Table 1

Chemical composition of the pancreas of the normal and growth retarded neonatal pig at birth (mean + SEM)

	Normal (n = 6)	IUGR (n = 5)
Pancreatic weight (g)	1.60 ± 0.10	0.57 ± 0.04*
Pancreatic Wt:body Wt (g/kg)	1.21 ± 0.05	0.96 ± 0.04*
Protein (mg/g tissue)	170 ± 4.7	160.8 ± 11
RNA (mg/g tissue)	6.20 ± 0.37	6.05 ± 0.33
DNA (mg/g tissue)	6.81 ± 0.71	7.66 ± 0.51
Total DNA (mg)	10.8 ± 0.9	4.3 ± 0.3*
Protein:DNA (mg/mg)	25.9 ± 1.7	21.0 ± 0.4
RNA:DNA ration (mg/mg)	0.93 ± 0.06	0.80 ± 0.06

* p < 0.05

Results presented in this table show that the pancreas was disproportionately smaller in IUGR piglets than that in normal piglets. The retardation of pancreatic development observed in the IUGR piglets was mainly due to hypoplasia less cell number as total pancreatic DNA content was lower in those animals. Histological examination showed reduction in all cell types.

EXAMPLE 2

Studies were performed in newborn piglets, that were raised for 24 hours following birth with a commercial infant milk formula (SMA Gold Cap; John Wyeth & Bro (NZ) Ltd) containing undetectable (<1 ng/ml) levels of IGF-I or IGF-II or with the same formula supplemented with either 2 µg/ml of recombinant human IGF-I or recombinant human IGF-II (provided Kabi Pharmacia AB, Sweden). 7 piglets recieved each treatment. The piglets were from 7 litters and each litter provided on one formula fed, one formula plus IGF-I fed and one formula plus IGF-II fed piglet. The piglets had statistically similar birth weights. After birth the piglets were fed by bottle 20 ml/kg every 2 hours for the first 12 hours, then 40 ml/kg every 4 hours thereafter until slaughter. Prior to slaughter the animals were injected with BDRU to enable calculation of cellular mitotic rate. The animals were thereafter slaughtered at 24 hours after birth for histological evaluation.

Table 2

Mean body-weight and weights and physical dimensions of digestive organs in 24 hour old piglets raised on an infant formula with or without addition of IGF-I or IGF-II. (n=7)

	Control	IGF-I	IGF-II
Birth Weight (kg)	1.286	1.317	1.295
Final Weight (kg)	1.318	1.328	1.320
Stomach weight (g/kg)#	5.02	5.11	4.99
Pancreas weight (g/kg)#	1.23	1.41**	1.37*
Small intestine			
Weight (g/kg)#	29	32	29
Length (cm/kg)#	310	293	323
Mitotic index (cells/crypt labelled)	6.93	9.09***	8.63***
Large intestine			
Weight (g/kg)#	6.2	6.3	6.4
Length (cm/kg)#	69	66	72

Adjusted for the birth weight.

* p < 0.05; ** p < 0.01; *** p < 0.001

Table 3

Chemical compositions of the pancreas and the proximal jejunal mucosa.

	Control	IGF-I	IGF-II
Pancreas:			
(n=7)			
Weight (g/kg)#	1.23	1.41**	1.37*
Protein (mg/kg)#	153	182**	182*
RNA (mg/kg)#	11.6	14.2*	13.5*
DNA (mg/kg)#	4.4	6.1*	5.9*
Protein: DNA	35.3	31.5	32.0
RNA: DNA ratio	2.67	2.43	2.40
(mg/mg)			
Proximal jejunal mucosa:			
(n=5)			
Weight (g/kg)#	5.712	6.754	6.147
Protein (mg/kg)#	583	623	559
RNA (mg/kg)#	28.9	33.7	31.0
DNA (mg/kg)#	25.0	30.4	28.1
Protein: DNA	23.0	20.5	20.0
RNA: DNA ratio	1.14	1.12	1.11
(mg/mg)			

Adjusted for the birth weight.

* p < 0.05

** p < 0.01

These observations provide clear evidence that in neonatal animals oral administration of IGF-I and IGF-II are selectively active to promote growth of the pancreas.

Histological examination showed the hyperplasia (increase of number of cells) of the pancreas involved the exocrine pancreas. The effect on the pancreas was particularly marked compared to other aspects of the gastrointestinal system. The increase in pancreatic size is due to an increase in cell number but not the cell size as the DNA content increases but protein:DNA ratio remained constant. These observations show clearly that oral IGF-I or IGF-II promotes pancreatic hyperplasia.

Light microscopy showed this was due to an increase in pancreatic exocrine cells dominant cell of the pancreas responsible for the production of fluids and enzymes necessary for absorption.

The data clearly show activity of oral administered IGF-I or IGF-II even in the presence of artificial milk.

Evidence suggests differential effects of IGF-I and IGF-II such that specific effects will be possible by selection of a suitable dose and preferential use of IGF-I or IGF-II or their admixture.

EXAMPLE 3

Further histological data was obtained from the histological examination of sections of the pancreases obtained for example 2.

The cellular features of the same pancreases from the control and the IGF-I and IGF-II treatment groups were therefore evaluated quantitatively. All cell counts related to the area of the visual field minus connective tissue, blood vessels etc. The features examined were

(1) with regard to all cells, total cells per unit area, mean area per cell and the BrdU (bromdeoxyuridine, a marker for DNA synthesis) mitotic index,

(2) with regard to endocrine cells, the numbers per unit area of insulin, glucagon and somatostatin secreting cells per unit area, and the total for the three types, and

(3) by difference, the number of exocrine cells per unit area. Endocrine cells of different types were identified by standard immunohistochemical techniques employing specific antibodies for insulin, glucagon and somatostatin. The total number of endocrine cells was determined in each case by adding the numbers for insulin, glucagon and somatostatin stained cells.

Results

1. There was a 14.6% increase in pancreatic weight with IGF-I ($P < 0.01$); associated with that was a 6 % increase in mean cell size and an 11 % increase in mitotic index. This suggests that both hypertrophy and hyperplasia was induced by IGF-I.
2. There was an 11.4% increase in pancreatic weight with IGF-II ($P < 0.05$); associated with this was a 5% increase in mean cell size but no increase in mitotic index. This suggests IGF-II induces hypertrophy alone.

The results from the histological examination are presented in Table 4.

Table 4Features of the pancreas ($\bar{x} \pm \text{SEM}$)

	Control	IGF-I	IGF-II
<u>Total cells</u>			
Cells/unit area (no/mm ²)	9942 \pm 493	9373 \pm 354	9443 \pm 347
Area/cell (μm^2 /cell)	102 \pm 5.4	108 \pm 4.7	107 \pm 3.5
BrdU index (no/mm ²)	14.8 \pm 3	16.5 \pm 2.7	14.9 \pm 1.8
<u>Endocrine cells</u>			
Insulin (no/mm ²)	692 \pm 73	741 \pm 68	633 \pm 112
Glucagon (no/mm ²)	1015 \pm 90	960 \pm 83	1065 \pm 108
Somatostatin (no/mm ²)	57 \pm 13	69 \pm 17	48 \pm 10
Total Endocrine cells (no/mm ²)	1765 \pm 109	1770 \pm 91	1746 \pm 174

CLAIMS

1. Use of IGF-I and/or IGF-II or effective analogues thereof for the manufacture of a medicament adopted for oral or any gastrointestinal route of administration for prevention or treatment of pancreatic disorders and insufficiency.
2. Use according to claim 1 in which the medicament promotes pancreatic growth.
3. Use according to claim 1 in which the medicament promotes pancreatic development in growth retarded children.
4. Use according to any of claims 1-3 in which human or animal IGF-I is used.
5. Use according to any of claims 1-3 in which human or animal IGF-II is used.
6. Use according to any claims 1-5 in which IGF-I and IGF-II may be given singly or in combination with each other or with other growth factors.
7. Composition comprising exogenous human or animal IGF-I and/ or IGF-II or effective analogues thereof comprising foodstuff for oral administration.
8. Composition according to claim 7 for prevention or treatment of pancreatic disorders comprising exogenous human or animal IGF-I and/ or IGF-II or effective analogues thereof.
9. Composition according to any of claims 7-8 comprising artificial or natural milk or colostrum.

10. Method for the prevention or treatment of pancreatic disorders and insufficiency by administration of a medicament adopted for oral or any gastrointestinal route comprising IGF-I and/or IGF-II or effective analogues thereof.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 93/00502

A. CLASSIFICATION OF SUBJECT MATTER

IPC5: A61K 37/36, A61K 37/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: A61K, C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO, A1, 9015142 (GROPEP PTY. LTD.), 13 December 1990 (13.12.90), see claim 29, page 4, lines 21-22 --	1-9
X	WO, A1, 9103253 (GREATER GLASGOW HEALTH BOARD), 21 March 1991 (21.03.91) -- It is pointed out that the basis of the A-category is that a known compound may in many countries be claimed by a product claim restricted to the first medical use and in many of these countries by a use claim for other medical indications (second medical indication). -- -----	1-9

☐ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
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Date of the actual completion of the international search

8 Sept 1993

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 93/00502

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>Dialog Information Services, file 155, Medline, Dialog accession no. 07566682, Medline accession no. 91085682, Young GP et al: "Insulin-like growth factors and the developing and mature rat small intestine: receptors and biological actions", Digestion 1990, 46 Suppl 2 p240-52</p> <p style="text-align: center;">-- -----</p>	1-9

INTERNATIONAL SEARCH REPORT

I. national application No.

PCT/SE 93/00502

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 10
because they relate to subject matter not required to be searched by this Authority, namely:
See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

30/07/93

International application No.
PCT/SE 93/00502

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A1- 9015142	13/12/90	AU-A- 5667590 EP-A- 0429586	07/01/91 05/06/91
WO-A1- 9103253	21/03/91	AU-A- 6358090 EP-A- 0490955	08/04/91 24/06/92